Chemical Studies on Crude Drug Processing. VIII.[1] On the Constituents of Rehmanniae Radix. (2): Absolute Stereostructures of Rehmaglutin C and Glutinoside Isolated from Chinese Rehmanniae Radix, the Dried Root of *Rehmannia glutinosa* LIBOSCH.

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A full account of the structure elucidation of an iridoid lactone, rehmaglutin C, and a chlorinated tricyclic iridoid glucoside, glutinoside, is presented. Rehmaglutin C (10) and glutinoside (11) were isolated from Chinese Rehmanniae Radix, the dried root of *Rehmannia glutinosa* LIBOSCH. from China, called Kan-jio in Japanese. The absolute stereostructures 10 and 11 have been determined on the basis of chemical and physicochemical evidence which included application of the exciton chirality method for the allylic benzoyl derivative.

Key words Rehmanniae Radix; iridoid lactone; iridoid glucoside; rehmaglutin C; glutinoside; exciton chirality method

During the course of our chemical characterization studies on the processing of Rehmanniae Radix,[2,3] we have been investigating the chemical constituents of Chinese Rehmanniae Radix (Kan-jio in Japanese), the dried root of *Rehmannia glutinosa* LIBOSCH. (Scrophulariaceae). We have so far isolated four iridoids named rehmaglutins A (**7**), B (**8**), C (**10**), and D (**9**) from the less polar fraction of Chinese Rehmanniae Radix, and reported the absolute stereostructures of rehmaglutins A (**7**), B (**8**) and D (**9**).[1,4] In continuing studies of the water-soluble portion of Chinese Rehmanniae Radix, we isolated a chlorinated iridoid glucoside, glutinoside (**11**),[5] three ionone glucosides, rehmaionosides A,[6] B,[6] and C,[6] and a monoterpene glucoside, rehmapicroside,[6] together with six known iridoid glycosides, catalpol (**1**), leonuride (**2**), monomelittoside (**3**), melittoside (**4**), rehmannioside D (**5**) and dihydrocornin (**6**).[3]

This paper presents a full account of the isolation of the glycosides and the structure elucidation of rehmaglutin C (**10**) and glutinoside (**11**).[7]

Rehmaglutin C (10) Rehmaglutin C (**10**) was obtained as a colorless oily substance. The chemical ionization mass

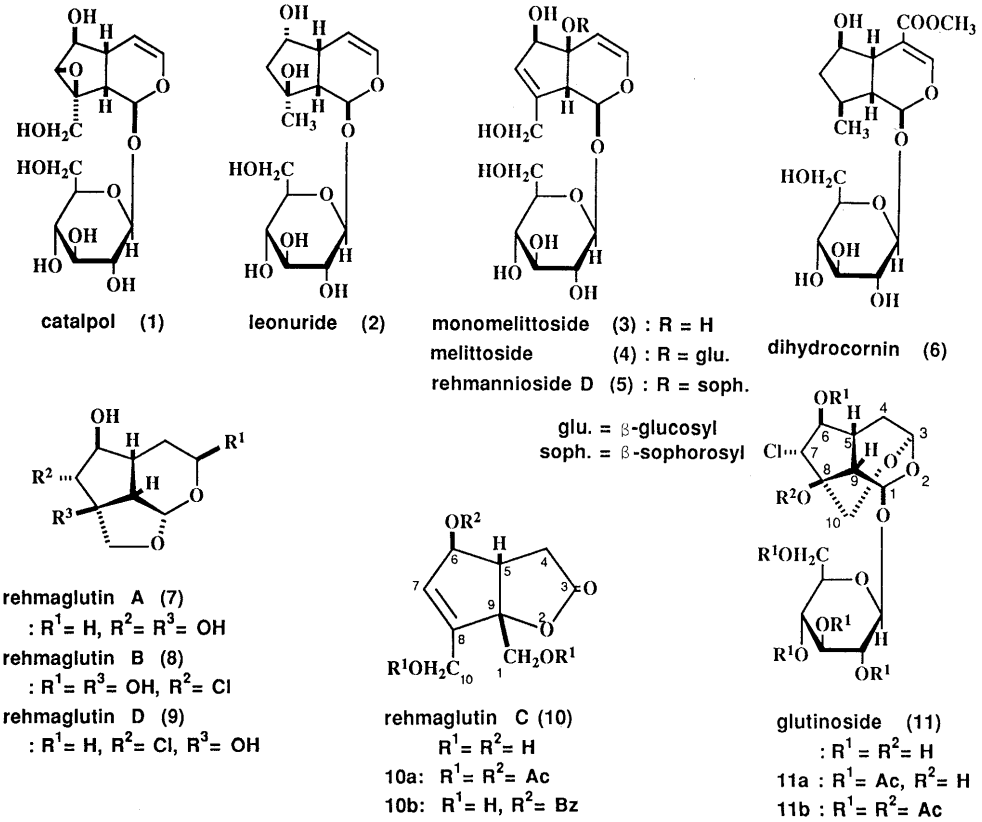

Chart 1

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spectrum (CI-MS) of **10** showed the quasimolecular ion peak at *m/z* 201 $(M+H)^+$ together with fragment ion peaks assignable to $(M+H-H_2O)^+$ (*m/z* 183) and $(M+H-2H_2O)^+$ (*m/z* 165). The high-resolution mass (high MS) measurement of **10** revealed the molecular formula of rehmaglutin C to be $C_9H_{12}O_5$. The infrared (IR) spectrum of **10** featured strong absorptions at 3340 and 1758 cm^{-1} due to hydroxyl groups and a γ-lactone group, respectively. The proton and the carbon-13 nuclear magnetic resonance (^{1}H- and ^{13}C-NMR) spectra of **10** showed the presence of one secondary hydroxyl group [δ 4.52 (br s, 6-H), δ_C 81.0 (6-C)], two primary hydroxyl groups [δ 3.70, 3.89 (both d, J=11.4 Hz, 1-H_2), 4.19 (br s, 10-H_2), δ_C 59.0 (1-C), 65.8 (10-C)] and a trisubstituted olefin moiety [δ 5.98 (br d, J=2.0 Hz, 7-H), δ_C 134.7 (7-C), 147.4 (8-C)].

The ^{1}H-NMR spectrum of the triacetate (**10a**), prepared by acetylation of **10** with acetic anhydride and pyridine, was assigned as shown in Table 1 on the basis of detailed decoupling experiments. Based on the comparisons of the ^{1}H- and ^{13}C-NMR data (Table 3) for **10a** with those for **10**, rehmaglutin C (**10**) was assumed to be the iridoid lactone derivative having 1,10-primary hydroxyl groups, a 6-secondary hydroxyl group and a 7,8-olefin moiety. Furthermore, the relative configuration of **10a** was clarified by nuclear Overhauser effect (NOE) experiments as depicted in Fig. 1.

In order to elucidate the absolute configuration of rehmaglutin C (**10**), the allylic benzoate method[8] was applied to 6-*O*-benzoylrehmaglutin C (**10b**), which was prepared from **10** through the following procedures: i) monomethoxytritylation of **10** with *p*-anisylchlorodiphenylmethane (MMTrCl) in pyridine to protect the 1,10-primary hydroxyl groups, ii) benzoylation with benzoyl chloride in pyridine and then iii) removal of the monomethoxytrityl groups with *p*-toluenesulfonic acid in methanol–tetrahydrofuran (THF). The ultraviolet (UV)

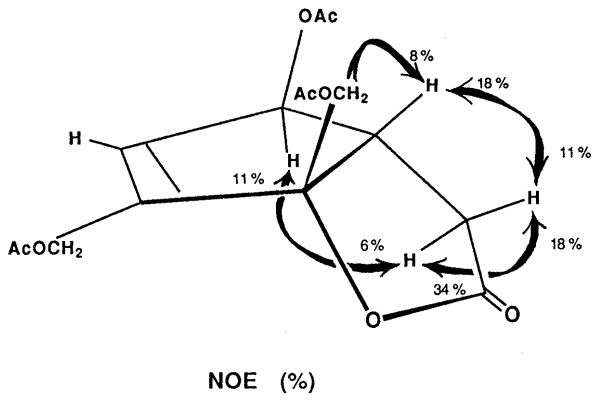


Fig. 1. NOE Correlations for **10a**

Table 1. ^{1}H-NMR Data[a] for **10a**, **12**, **12a** and **12b**

	10a	**12**	**12a**	**12b**
H-1	4.25 (d, J=11.8) 4.47 (d, J=11.8)	4.93 (br s)	5.02 (br s)	5.08 (br s)
H-3		4.48 (t, J=6.2)	4.34 (t, J=5.3)	4.36 (t, J=5.2)
H-4	2.65 (dd, J=5.1, 18.8, α-H) 3.05 (dd, J=11.1, 18.8, β-H)	1.62—2.09	1.60—1.80	1.65—1.79
H-5	2.86 (ddd, J=1.8, 5.1, 11.1)	2.05—2.30	2.30—2.50	2.60—3.00
H-6	5.35 (br s)	—[b]	4.95 (d, J=10.2)	4.54 (d, J=9.7)
H-7	6.07 (dd, J=2.0)	4.08 (dd, J=1.8, 10.3)	4.30 (dd, J=1.3, 10.2)	4.74 (br d, J=9.7)
H-9		2.44 (br d, J=9.0)	2.30—2.50	2.60—3.00
H-10	4.76 (dd, J=2.0, 13.3) 4.80 (dd, J=2.0, 13.3)	3.72 (dd, J=1.8, 10.2, β-H) 4.25 (d, J=10.2, α-H)	3.77 (dd, J=1.3, 10.0, β-H) 4.31 (d, J=10.0, α-H)	3.89 (dd, J=1, 10.0, β-H) 4.62 (d, J=10.0, α-H)

a) The spectrum of **10a** was measured at 500 MHz in $CDCl_3$ and those of **12**, **12a** and **12b** at 90 MHz in $CDCl_3$. Chemical shifts are in δ and J values are in Hz. *b*) The signal due to 6-H overlapped the signal of the methoxyl methyls.

Table 2. ^{1}H-NMR Data for **11a** and **11b** at 500 MHz (in $CDCl_3$)

	11a	**11b**
H-1	5.55 (d, J=1.6)	5.55 (d, J=1.7)
H-3	5.32 (d, J=2.9)	5.34 (d, J=2.9)
H-4	2.16 (dd, J=2.9, 15.4, α-H) 1.98 (dd, J=9.7, 15.4, β-H)	2.17 (dd, J=2.9, 14.2, α-H) 1.99 (dd, J=10.1, 14.2, β-H)
H-5	2.22 (ddd, J=3.0, 9.7, 10.2)	2.26 (ddd, J=2.8, 10.1, 10.3)
H-6	4.93 (dd, J=3.0, 7.8)	4.91 (dd, J=2.8, 8.0)
H-7	4.28 (d, J=7.8)	5.30 (d, J=8.0)
H-9	2.66 (br d, J=10.2)	3.51 (br d, J=10.3)
H-10	3.67 (d, J=12.2, β-H) 4.07 (d, J=12.2, α-H)	3.87 (dd, J=1.8, 13.0, β-H) 4.26 (d, J=13.0, α-H)
H-1′	4.94 (d, J=7.7)	4.93 (d, J=7.7)
H-2′	4.99 (dd, J=7.7, 8.9)	4.98 (dd, J=7.7, 9.0)
H-3′	5.22 (dd, J=8.9, 10.1)	5.22 (dd, J=9.0, 10.1)
H-4′	5.11 (dd, J=9.7, 10.1)	5.11 (dd, J=10.1, 9.7)
H-5′	3.73 (ddd, J=2.1, 4.0, 9.7)	3.73 (ddd, J=1.9, 3.9, 9.7)
H-6′	4.13 (dd, J=2.1, 13.2) 4.31 (dd, J=4.0, 13.2)	4.14 (dd, J=1.9, 13.2) 4.32 (dd, J=3.9, 13.2)

Table 3. ^{13}C-NMR Data for Rehmaglutin C (**10**), the Triacetate (**10a**), Glutinoside (**11**), and the Derivatives (**11a**, **11b**, **13**, **12**, **12a**, **12b**)

	10[a]	**10a**[a]	**12**[a]	**12a**[a]	**12b**[a]	**11**[b]	**11a**[b]	**11b**[b]	**13**[b]
C-1	59.0	60.5	104.9	104.6	104.7	94.6	94.8	94.8	94.3
C-3	177.0	175.2	107.8	107.5	107.5	92.6	93.5	93.1	93.1
C-4	35.4	34.8	32.8	32.4	32.5	33.9	33.5	33.8	29.4
C-5	50.5	47.6	40.1	38.6	38.4	35.5	33.5	33.8	29.8
C-6	81.0	83.4	75.3	79.3	80.0	84.3	86.4	86.2	61.8
C-7	134.7	133.2	79.9	71.6	67.0	75.6	70.3	64.5	58.6
C-8	147.4	145.1	86.1	85.9	91.0	79.3	79.1	86.0	79.3
C-9	99.6	94.9	57.8	57.7	55.8	47.5	47.4	43.1	47.5
C-10	65.8	66.7	73.9	73.5	71.9	61.9	62.1	59.9	64.4
C-1′						98.6	96.5	96.3	98.5
C-2′						74.2	71.5	71.8	74.3
C-3′						77.9	73.2	73.3	77.8[c]
C-4′						70.9	68.9	69.0	70.9
C-5′						77.9	72.3	72.5	77.9[c]
C-6′						61.9	62.1	62.0	61.5

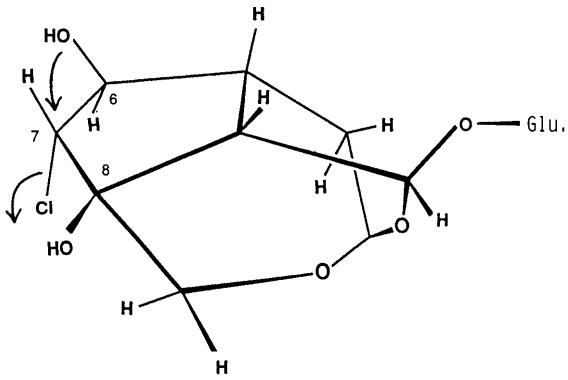
Measured at 22.5 MHz in *a*) d_6-acetone or *b*) pyridine-d_5. Chemical shifts are in δ_C. *c*) The assignments may be interchanged.

and IR spectra of **10b** showed the presence of the benzoyl group, and the ^{1}H-NMR spectrum exhibited a signal due to the benzoyloxy-bearing methine proton [δ 5.64 (br s, 6-H)]. The circular dichroism (CD) spectrum of **10b** gave a negative first Cotton curve ($[\theta]_{228}$ −9500), which indicated the 6*S* configuration of **10b**. Thus, the absolute stereostructure of rehmaglutin C (**10**) was determined to be as shown.

Glutinoside (11) Glutinoside (**11**), obtained as a hygroscopic white powder, was shown to possess a chlorine atom by the positive Beilstein test. The secondary ionization mass spectrum (SI-MS) of **11** showed pairs of isotope ion peaks at *m/z* 399 and 401 due to $(M+H)^+$, at *m/z* 421 and 423 due to $(M+Na)^+$ and at *m/z* 491 and 493 due to $(M+H+glycerol)^+$. The high SI-MS measurement for the quasimolecular ion peak $(M+H)^+$ of **11** revealed the molecular formula to be $C_{15}H_{23}ClO_{10}$. The ^{1}H- and ^{13}C-NMR data for **11** suggested the presence of two acetal groups [δ 5.23, 5.63 (both br s, 3, 1-H), δ_C 94.6, 92.6 (1, 3-C)], one secondary and one tertiary hydroxyl group [δ_C 84.3, 79.3 (6,8-C)], and the β-D-glucopyranosyl moiety.

Ordinary acetylation of **11** gave the pentaacetate (**11a**) and the hexaacetate (**11b**). Detailed ^{1}H-NMR decoupling experiments enabled us to make complete assignments of the signals of **11a** and **11b** (Table 2). Comparisons of the ^{1}H- and ^{13}C-NMR data (Table 3) for **11**, **11a** and **11b** with those for rehmaglutins A (**7**), B (**8**) and D (**9**) indicated the presence of 6,8-dihydroxyl groups and a 7-chloro substituent in the tricyclic iridoid skeleton.

Methanolysis of **11** with 9% HCl–dry methanol at room temperature yielded methyl glucopyranoside and the acetal (**12**), which provided the monoacetate (**12a**) and the diacetate (**12b**) by ordinary acetylation. Comparisons of the ^{1}H- and ^{13}C-NMR data (Tables 1, 3) for **12**, **12a** and **12b** with those for rehmaglutin B (**8**) and D (**9**) led us to presume the structure **12**, having 6,8-dihydroxyl groups, a 7-chloro group and a 1,10-oxide ring.[9] Finally, methanolysis of catalpol (**1**) under the same reaction conditions as described for the methanolysis of **11** gave **12** in 35% yield together with several minor products. On the other hand, treatment of **11** with 10% aqueous HCl provided rehmaglutin B (**8**) in 54% yield. Based on this evidence, glutinoside (**11**) has been assumed to be the tricyclic iridoid glucopyranoside having 6,8-dihydroxyl groups and a 7-chloro residue.



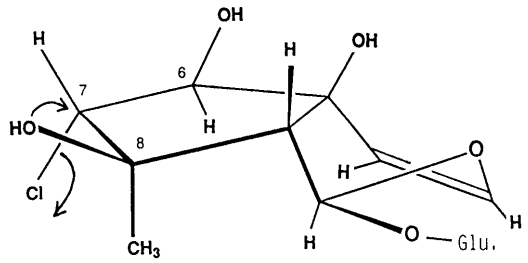


Fig. 2. Conformations of Glutinoside (**11**) and Linalioside (**14**)

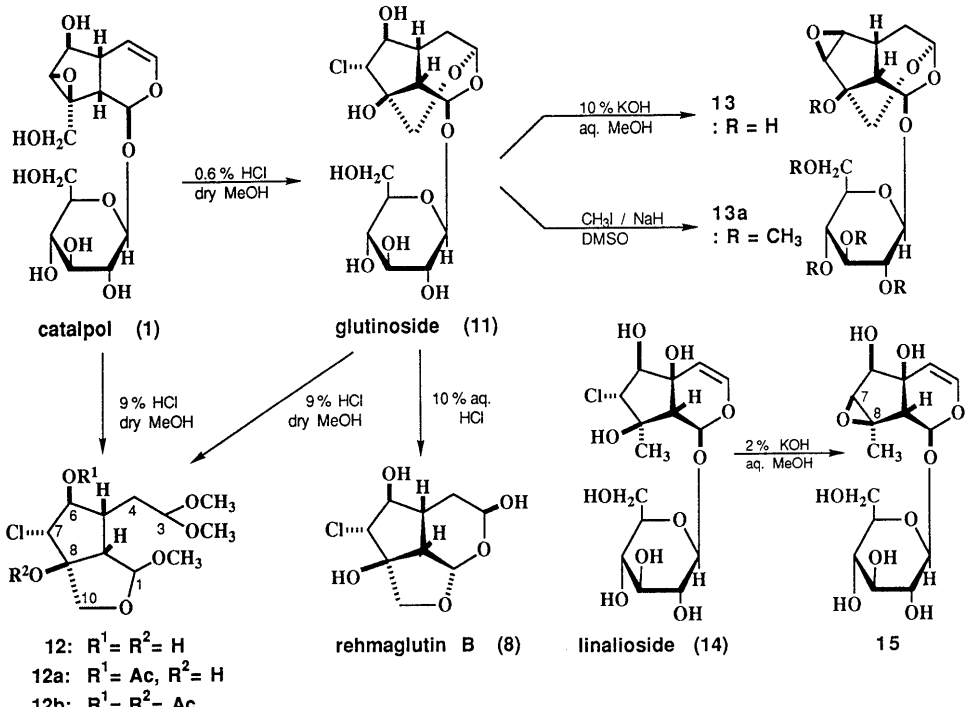


Chart 2

Alkaline treatment of **11** with 10% aqueous KOH under reflux afforded the 6β,7β-epoxide (**13**) in 53% yield. Previously, we reported that mild alkaline treatment of linalioside (**14**), isolated from the root of *Linaria japonica*, provided the 7β,8β-epoxide derivative (**15**).[10] However, the alkaline treatment of **11** under the same reaction conditions as those reported for **14** gave no product, and the starting **11** was recovered. Based on a comparison with the conformations of **11** and **14** using Dreiding models, as shown in Fig. 2, it was presumed that the deformed conformation (i) of **11** caused by the 3,10-oxide ring favored attack of the 6β-hydroxyl group, while the conformation (ii) of **14** favored attack of the 8β-hydroxyl group. Methylation of **11** with CH_3I/DMSO/NaH[11] furnished the penta-*O*-methyl derivative (**13a**), which yielded methyl 2,3,4,6-tetra-*O*-methylglucopyranoside upon methanolysis. Finally, the absolute stereostructure of glutinoside (**11**) was substantiated by chemical correlation with catalpol (**1**). Namely, treatment of **1** with 0.6% HCl–dry methanol at room temperature provided **11**, formed *via* cleavage of the 7,8-epoxide ring and acetal formation between C-3 and C-10. Thus, the structure of glutinoside (**11**) was determined to be as shown.

Experimental

The instruments used to obtain physical data and the experimental conditions for chromatography were the same as described in our previous paper.[1]

Isolation of Rehmaglutin C (10) Rehmaglutin C (**10**) was isolated as described in our previous paper.[1] Rehmaglutin C (**10**): colorless oil, $[\alpha]_D^{24}$ −51.4° (c=0.71, MeOH). High MS: Found 200.069. Calcd for $C_9H_{12}O_5$ (M^+) 200.069. IR ν_{max}^{film} cm^{-1}: 3340, 1758. ^{1}H-NMR (90 MHz, acetone-d_6) δ: 3.70, 3.89 (1H each, both d, J=11.4 Hz, 1-H_2), 4.19 (2H, br s, 10-H_2), 4.52 (1H, br s, 6-H), 5.98 (1H, br d, J=*ca.* 2 Hz, 7-H). ^{13}C-NMR: see Table 3. CI-MS m/z (%): 201 [$(M+H)^+$, 12], 183 [$(M+H-H_2O)^+$, 100], 165 [$(M+H-2H_2O)^+$, 86].

Isolation of Glutinoside (11), Catalpol (1), Leonuride (2), Monomelittoside (3), Melittoside (4), Rehmannioside D (5), Dihydrocornin (6), Rehmaionosides A, B, and C, and Rehmapicroside The glycoside mixture (43 g), obtained from Chinese Rehmanniae Radix (3 kg) as reported previously,[1] was fractionated by reversed-phase silica gel column chromatography [Bondapak C_{18} 500 g, H_2O, H_2O–MeOH (10:1→5:1→2:1) as the eluent] to furnish four fractions. Evaporation of the solvent from each fraction under reduced pressure gave fr. 1 (24.1 g, eluted by H_2O), fr. 2 [8.2 g, H_2O–MeOH (10:1)], fr. 3 [2.1 g, H_2O–MeOH (5:1)] and fr. 4 [2.7 g, H_2O–MeOH (2:1)].

Fraction 1 (24.1 g) was subjected to normal-phase column chromatography [SiO_2 500 g, $CHCl_3$–MeOH–H_2O (65:35:10), using the lower phase] to give glutinoside (**11**, 1.50 g), catalpol (**1**, 1.05 g) and monomelittoside (**3**, 28 mg). Fraction 2 (8.2 g) was subjected successively to normal-phase column chromatography [SiO_2 500 g, $CHCl_3$–MeOH–H_2O (6:4:1)] and reversed-phase silica gel column chromatography [Bondapak C_{18}, 100 g, H_2O–MeOH (10:1)] to give leonuride (**2**, 78 mg), melittoside (**4**, 562 mg), rehmannioside D (**5**, 920 mg) and dihydrocornin (**6**, 27 mg). Fraction 3 (2.1 g) was purified by normal-phase column chromatography [SiO_2 100 g, $CHCl_3$–MeOH–H_2O (6:4:1)] to give rehmapicroside (50 mg). Fraction 4 (2.7 g) was purified by normal-phase column chromatography [SiO_2 100 g, $CHCl_3$–MeOH–H_2O (65:35:10), lower phase] to afford rehmaionoside C (14 mg) and a mixture of rehmaionosides A and B, which was further purified by HPLC (Zorbax ODS, H_2O–MeOH) to give rehmaionoside A (55 mg) and B (171 mg).

Catalpol (**1**) was shown to be identical with an authentic sample which was isolated previously from Japanese Rehmanniae Radix[2] by mixed melting point determination, and TLC [$CHCl_3$–MeOH–H_2O (6:4:1), *n*-BuOH–AcOH–H_2O (4:1:1)], IR (KBr), and ^{1}H-NMR (CD_3OD) comparisons. Leonuride (**2**), monomelittoside (**3**), melittoside (**4**), rehmannioside D (**5**) and dihydrocornin (**6**) were identified by comparing their physical data with reported values. In order to confirm these identifications, the acetylated derivatives of the iridoid glycosides were prepared under the same acetylation conditions as those reported, and identified. Leonuride (**2**)[12,13]: IR (KBr), ^{1}H- (pyridine-d_5) and ^{13}C-NMR (D_2O, pyridine-d_5); leonuride hexaacetate (prepared from **2**)[12,13]: mp 172—174 °C, $[\alpha]_D$, ^{1}H- ($CDCl_3$) and ^{13}C-NMR ($CDCl_3$); monomelittoside (**3**)[14]: IR (KBr), ^{1}H- (D_2O) and ^{13}C-NMR (D_2O); melittoside (**4**)[12]: IR (KBr), ^{1}H- (D_2O) and ^{13}C-NMR (D_2O): melittoside decaacetate (prepared from **4**)[12]: IR (CCl_4), ^{1}H- ($CDCl_3$) and ^{13}C-NMR ($CDCl_3$); rehmannioside D (**5**)[12]: IR (KBr), ^{1}H- (D_2O) and ^{13}C-NMR (D_2O); rehmannioside D tridecaacetate (prepared from **5**),[12] IR (CCl_4), ^{1}H- ($CDCl_3$) and ^{13}C-NMR ($CDCl_3$); dihydrocornin (**6**): $[\alpha]_D$, IR (KBr), ^{1}H-NMR (D_2O, pyridine-d_5) and ^{13}C-NMR (pyridine-d_5); dihydrocornin pentaacetate (prepared from **6**)[14]: IR (CCl_4), ^{1}H- ($CDCl_3$) and ^{13}C-NMR (pyridine-d_5).

Glutinoside (**11**): Hygroscopic amorphous powder, $[\alpha]_D^{20}$ −79.2° (c=2.49, MeOH). High SI-MS: Found 399.106. Calcd for $C_{15}H_{24}{}^{35}ClO_{10}$ $(M+H)^+$ 399.106. IR ν_{max}^{KBr} cm^{-1}: 3388, 2922, 1047. ^{1}H-NMR (90 MHz, pyridine-d_5) δ: 5.23 (1H, br s, 3-H), 5.48 (1H, d, J=7.4 Hz, 1′-H), 5.63 (1H, br s, 1-H). ^{13}C-NMR: see Table 3. SI-MS (Xe^+, glycerol matrix) m/z: 399, 401 $(M+H)^+$, 421, 423 $(M+Na)^+$, 491, 493 (M+H+glycerol)$^+$.

Acetylation of Rehmaglutin C (10) A solution of **10** (10 mg) in pyridine (1.0 ml) was treated with Ac_2O (1.0 ml) and the mixture was stirred at room temperature (19 °C) under an N_2 atmosphere for 12 h, then poured into ice-water. The whole was extracted with AcOEt. The AcOEt extract was washed with 2 N HCl, water, aqueous saturated $NaHCO_3$ and brine, and then dried over $MgSO_4$. The AcOEt layer was concentrated under reduced pressure to give **10a** (16 mg).

10a: Colorless oil, $[\alpha]_D^{20}$ −42.6° (c=0.82, MeOH). High-MS: Found 326.102. Calcd for $C_{15}H_{18}O_8$ (M^+) 326.100. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1775, 1739, 1235. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 2.08, 2.11, 2.13 (3H each, all s, $CH_3CO\times3$), and other signals as given in Table 1. NOE (%): as shown in Fig. 1. ^{13}C-NMR (22.5 MHz, acetone-d_6) δ_C: 21.1 (2C), 21.3, 171.0 (3C) and other data as given in Table 3; ($CDCl_3$): 20.7 (q, $\underline{C}H_3CO\times2$), 20.8 (q, $\underline{C}H_3CO$), 34.0 (t, 4-C), 46.6 (d, 5-C), 59.3 (t, 1-C), 65.5 (t, 10-C), 82.0 (d, 6-C), 94.9 (s, 9-C), 131.8 (d, 7-C), 143.6 (s, 8-C), 170.1 (s, $CH_3\underline{C}O\times2$), 170.5 (s, $\underline{C}H_3CO$), 174.3 (s, 3-C).

Conversion of Rehmaglutin C (10) to 10b A solution of **10** (4 mg) in pyridine (1.5 ml) was treated with MMTrCl (32 mg) and the mixture was stirred at room temperature (22 °C) under an N_2 atmosphere for 48 h. It was then treated with BzCl (0.025 ml) and the mixture was stirred at room temperature (23°C) under an N_2 atmosphere for 8 h. This mixture was poured into ice-water and the whole was extracted with AcOEt. The AcOEt extract was washed with 2 N HCl, aqueous saturated $NaHCO_3$, and brine, then dried over $MgSO_4$. Removal of the solvent under reduced pressure gave a product, which was dissolved in MeOH (1.5 ml)–THF (0.7 ml), and the solution treated with *p*-TsOH·H_2O (trace). The reaction solution was stirred at room temperature (24 °C) under an N_2 atmosphere for 1 h and then poured into ice-water. The whole was extracted with AcOEt and the AcOEt extract was worked up in the same manner as described above to give a product, which was purified by column chromatography [SiO_2 1 g, $CHCl_3$–MeOH (20:1)] to furnish **10b** (2 mg).

10b: Colorless oil, $[\alpha]_D^{21}$ −82.4° (c=0.10, MeOH). High-MS: Found 304.097. Calcd for $C_{16}H_{16}O_6$ (M^+) 304.095. UV λ_{max}^{MeOH} nm (ε): 231 (6700). CD (MeOH): $[\theta]_{228\,nm}$ −9500 (neg. max.) IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400, 2920, 1769, 1713, 1599. ^{1}H-NMR: (90 MHz, acetone-d_6) δ: 3.58, 3.65 (1H each, both d, J=11.3 Hz, 1-H_2), 4.30 (2H, br s, 10-H_2), 5.64 (1H, br s, 6-H), 6.15 (1H, d, J=2.2 Hz, 7-H), 7.53—7.62 (3H, m), 7.97—8.08 (2H, m). CI-MS m/z (%): 305 [$(M+H)^+$, 22], 183 [$(M+H-C_6H_5COOH)^+$, 26].

Acetylation of Glutinoside (11) Giving 11a and 11b A solution of **11** (140 mg) in pyridine was treated with Ac_2O (3.0 ml) and the mixture was stirred at room temperature (18 °C) under an N_2 atmosphere for 3 h, then poured into ice-water. The whole was extracted with AcOEt. Work-up of the AcOEt extract in the usual manner gave a product (213 mg), which was purified by column chromatography [SiO_2 10 g, benzene–acetone (8:1)] to furnish **11a** (195 mg) and **11b** (18 mg).

11a: mp 185—186 °C (colorless prisms from MeOH), $[\alpha]_D^{20}$ −18.5° (c=0.81, $CHCl_3$). *Anal.* Calcd for $C_{25}H_{33}ClO_{15}$: C, 49.31; H, 5.46; Cl, 5.85. Found: C, 49.34; H, 5.46; Cl, 5.34. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3483, 2940, 1755, 1036. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 2.01, 2.10, 2.11 (3H each, all s), 2.03 (6H, s) (OAc×5) and other signals as given in Table 2. ^{13}C-NMR (22.5 MHz, pyridine-d_5) δ_C: 20.4 ($\underline{C}H_3CO\times5$), 169.7, 170.1, 170.3, 170.7, 170.8 ($CH_3\underline{C}O\times5$) and other data as given in Table 3. SI-MS (Xe^+, glycerol matrix) m/z: 609, 611 $(M+H)^+$, 701, 703

$(M+H+glycerol)^+$.

11b: mp 150—153 °C (colorless prisms from MeOH), $[\alpha]_D^{20}$ −34.4° ($c=1.60$, $CHCl_3$). *Anal.* Calcd for $C_{27}H_{35}ClO_{16}$: C, 49.81; H, 5.42; Cl, 5.45. Found: C, 49.93; H, 5.47; Cl, 5.27. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 2942, 1750, 1036. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 2.01, 2.08 (3H each, both s), 2.03, 2.11 (6H each, both s) ($CH_3CO\times6$) and other signals as given in Table 2. ^{13}C-NMR (22.5 MHz, pyridine-d_5) δ_C: 20.4 (5C), 21.6 ($\underline{C}H_3CO\times6$), 169.5, 169.7, 170.0, 170.2, 170.3, 170.6 ($CH_3\underline{C}O\times6$) and other data as given in Table 3.

Methanolysis of Glutinoside (11) A solution of **11** in 9% HCl–dry MeOH (1.0 ml) was stirred at room temperature (18°C) under an N_2 atmosphere for 4 h. After cooling, it was neutralized with Ag_2CO_3 and the product was shown by TLC [$CHCl_3$–MeOH–H_2O (65 : 35 : 10, lower phase), *n*-BuOH–AcOH–H_2O (4 : 1 : 5, upper phase)] to contain methyl glucoside. The reaction mixture was then filtered to remove the inorganic material and the filtrate was evaporated under reduced pressure to yield a product (22 mg). A small part (2 mg) of the product was dissolved in pyridine (0.1 ml) and the solution was treated with *N,O*-bis(trimethylsilyl)-trifluoroacetamide (0.2 ml) for 1 h, then analyzed by GLC to identify trimethylsilyl (TMS) derivatives of methyl glucopyranoside [GLC: 5% silicone SE-30 on Uniport HP (60—80 mesh); 3 mm × 2 m glass column; column temperature 170 °C; N_2 flow rate 40 ml/min; TMS-methyl glucoside 10 min 33 s, 11 min 28 s]. The product (20 mg) was purified by column chromatography [SiO_2 1 g, $CHCl_3$–MeOH (50 : 1)] to furnish **12** (6 mg).

12: Colorless oil, $[\alpha]_D^{20}$ +31.8° ($c=1.24$, MeOH). High-MS: Found 265.084. Calcd for $C_{11}H_{18}ClO_5$ (M^+-OCH_3) 265.084. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3411, 2929, 1068. ^{1}H-NMR (90 MHz, acetone-d_6) δ: 3.29 (6H, s), 3.31 (3H, s), ($OCH_3\times3$) and other signals as given in Table 1. ^{13}C-NMR: (22.5 MHz, acetone-d_6) δ_C: 53.4, 54.3, 55.1 ($OCH_3\times3$) and other data as given in Table 3. CI-MS *m/z* (%): 297 (1), 299 (0.3) $(M+H)^+$, 265 (100), 267 (34) $(M+H-CH_3OH)^+$, 233 (98), 235 (34) $(M+H-2CH_3OH)^+$.

Acetylation of 12 A solution of **12** (16 mg) in pyridine (0.5 ml) was treated with Ac_2O (0.5 ml) and the mixture was stirred at room temperature (18 °C) under an N_2 atmosphere for 5 h, then poured into ice-water. The whole was extracted with AcOEt. Work-up of the AcOEt extract in the usual manner gave the product (20 mg), which was purified by column chromatography [SiO_2 1 g, *n*-hexane–AcOEt (2 : 1)] to furnish **12a** (8 mg) and **12b** (11 mg).

12a: Colorless oil, $[\alpha]_D^{20}$ +40.7° ($c=0.79$, MeOH). High-MS: Found 307.096. Calcd for $C_{13}H_{20}ClO_6$ $(M-OCH_3)^+$ 307.095. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3444, 2934, 1748, 1230, 1040. ^{1}H-NMR (90 MHz, acetone-d_6) δ: 2.09 (3H, s, CH_3CO), 3.28, 3.29, 3.31 (3H each, all s, $OCH_3\times3$) and other signals as given in Table 1. ^{13}C-NMR (22.5 MHz, acetone-d_6) δ_C: 21.3 ($\underline{C}H_3CO$), 53.8, 54.6, 55.2 ($OCH_3\times3$), 171.4 ($CH_3\underline{C}O$) and other data as given in Table 3. CI-MS *m/z* (%): 307 (24), 309 (8) $(M+H-CH_3OH)^+$, 275 (7), 277 (3) $(M+H-2CH_3OH)^+$.

12b: Colorless oil, $[\alpha]_D^{20}$ +49.1° ($c=1.03$, MeOH). High-MS: Found 349.103. Calcd for $C_{15}H_{22}ClO_7$ $(M-OCH_3)^+$ 349.105. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 2950, 1747, 1228, 1040. ^{1}H-NMR (90 MHz, acetone-d_6) δ: 2.07, 2.10 (3H each, both s, $CH_3CO\times2$), 3.29, 3.30, 3.33 (3H each, all s, $OCH_3\times3$) and other signals as given in Table 1. ^{13}C-NMR (22.5 MHz, acetone-d_6) δ_C: 21.2, 21.9 ($\underline{C}H_3CO\times2$), 53.9, 54.7, 55.4 ($OCH_3\times3$), 170.9, 171.3 ($CH_3\underline{C}O\times2$) and other data as given in Table 3.

Methanolysis of Catalpol (1) Giving 12 A solution of **1** (70 mg) in 9% HCl–dry MeOH (2.0 mg) was stirred at room temperature (18 °C) under an N_2 atmosphere for 12 h, then neutralized with Ag_2CO_3 and filtered. After removal of the solvent from the filtrate under reduced pressure, the product was purified by column chromatography [SiO_2 1 g, $CHCl_3$–MeOH (50 : 1)] to furnish **12** (20 mg), which was shown to be identical with an authentic sample, which had previously been prepared by methanolysis of glutinoside (**11**), by TLC [$CHCl_3$–MeOH–H_2O (7 : 3 : 1, lower phase), $CHCl_3$–MeOH (10 : 1), benzene–acetone (3 : 1)], IR ($CHCl_3$) and ^{1}H-NMR acetone-d_6) comparisons.

Conversion of Glutinoside (11) to Rehmaglutin B (8) A solution of **11** (32 mg) in 10% aqueous HCl (2.0 ml) was stirred at room temperature (19 °C) for 22 h, then neutralized with Dowex 1 × 2 (OH^- form), and the resin was removed by filtration. The filtrate was concentrated under reduced pressure to yield a product (36 mg), which was purified by column chromatography [SiO_2 1 g, $CHCl_3$–MeOH (10 : 1)] to furnish **8** (10 mg), which was shown to be identical with authentic rehmaglutin B, which had been isolated previously from Chinese Rehmanniae Radix, by TLC [$CHCl_3$–MeOH–H_2O (7 : 3 : 1, lower phase), $CHCl_3$–MeOH (10 : 1), benzene–acetone (2 : 1)], IR (KBr) and ^{1}H-NMR (acetone-d_6) comparisons.

Alkaline Treatment of Glutinoside (11) A solution of **11** (160 mg) in MeOH (10 ml) was treated with 20% aqueous KOH (10 ml) and heated under reflux for 2.5 h. The reaction mixture was neutralized with Dowex 50W × 8 (H^+ form) and the resin was removed by filtration. The filtrate was concentrated under reduced pressure to yield a product (198 mg), which was purified by column chromatography [SiO_2 2 g, $CHCl_3$–MeOH–H_2O (65 : 35 : 10, lower phase)] to furnish **13** (77 mg).

13: mp 139—140 °C (colorless prisms from $CHCl_3$–MeOH), $[\alpha]_D^{20}$ −46.2° ($c=0.87$, MeOH). *Anal.* Calcd for $C_{15}H_{22}O_{10}\cdot2H_2O$: C, 45.23; H, 6.58. Found: C, 45.99; H, 6.51. IR ν_{max}^{KBr} cm^{-1}: 3400, 2920, 1073. ^{1}H-NMR (90 MHz, D_2O) δ: 5.20 (1H, br s, 3-H), 5.48 (1H, d, $J=2.4$ Hz, 1-H). ^{13}C-NMR: see Table 3.

A solution of **11** (20 mg) in 2% KOH–aqueous MeOH (5 ml) was heated at 40 °C for 1 h, neutralized with Dowex 50W × 8 (H^+ form), then filtered. After removal of the solvent from the filtrate, the product was purified by column chromatography [SiO_2 500 mg, $CHCl_3$–MeOH–H_2O (65 : 35 : 10, lower phase)] to recover **11** (12 mg), which was identical with authentic glutinoside by TLC [$CHCl_3$–MeOH–H_2O (6 : 4 : 1), *n*-BuOH–AcOH–H_2O (4 : 1 : 1)] comparison.

Methylation of Glutinoside (11) A solution of **11** (13 mg) in DMSO (1.0 ml) was treated with dimsyl carbanion (4 ml) and the mixture was stirred at 18°C under an N_2 atmosphere for 1 h. It was then treated with CH_3I (4 ml) with stirring in the dark for 12 h. The reaction mixture was poured into ice-water and the whole was extracted with AcOEt. The AcOEt extract was washed with 10% aqueous $Na_2S_2O_3$, and saturated saline, then dried over $MgSO_4$. Work-up of the AcOEt phase in the usual manner gave a product (15 mg) which was purified by column chromatography [SiO_2 1 g, benzene–acetone (10 : 1)] to furnish **13a** (13 mg).

13a: Colorless oil, $[\alpha]_D^{20}$ −12.4° ($c=0.80$, $CHCl_3$). *Anal.* Calcd for $C_{20}H_{32}O_{16}$: C, 55.55; H, 7.46. Found: C, 55.27; H, 7.57. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 2930, 1101. ^{1}H-NMR (90 MHz, acetone-d_6) δ: 3.32, 3.41, 3.48, 3.52, 3.57 (3H each, all s, $OCH_3\times5$), 4.67 (1H, d, $J=7.9$ Hz, 1′-H), 5.24 (1H, t, $J=1.1$ Hz, 3-H), 5.42 (1H, d, $J=1.8$ Hz, 1-H).

Conversion of Catalpol (1) to Glutinoside (11) A solution of **1** (50 mg) in 0.6% HCl–dry MeOH (1.2 ml) was stirred at room temperature (19 °C) under an N_2 atmosphere for 12 h, then neutralized with Dowex 1 × 2 (OH^- form), and the resin was removed by filtration. After removal of the solvent from the filtrate under reduced pressure, the product (58 mg) was purified by column chromatography [SiO_2 1 g, $CHCl_3$–MeOH–H_2O (6 : 4 : 1)] to give **11** (30 mg), which was shown to be identical with authentic glutinoside by TLC [$CHCl_3$–MeOH–H_2O (6 : 4 : 1), *n*-BuOH–AcOH–H_2O (4 : 1 : 1)], IR (KBr), ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (pyridine-d_5) comparisons.

References and Notes

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